Case Report

Human Umbilical Cord Mesenchymal Stem Cell Transplantation for the Treatment of Chronic Discogenic Low Back Pain

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Background: Chronic low back pain is one of the major causes of disability and thus has a major socioeconomic impact. Intervertebral disc degeneration is the main cause of chronic low back pain. Treatment for chronic discogenic low back pain has traditionally been limited to either conservative management or surgical fusion. If conservative treatment fails, then surgical fusion is commonly considered. Current treatments are limited to treat the symptoms and not the underlying biologic alterations of the disc.

Objective: Human umbilical cord tissue-derived mesenchymal stem cells (HUC-MSCs) contain stem cells and possess the ability to regenerate degenerative discs. Based on the results of previous in vitro and animal experiments, we conducted a preliminary study to test the feasibility and safety and to obtain an early indication for the therapeutic value of HUC-MSC transplantation in patients with chronic discogenic low back pain.

Study Design: This is the first study involving treatment of chronic low back pain using HUC-MSC transplantation.

Setting: The study was performed at a spine center in China.

Methods: Two patients with chronic discogenic low back pain were treated with HUC-MSC transplantation. An 11-point visual analog scale (VAS, 0 – 10) and Oswestry Disability Index (ODI, 0 – 100) were used to assess the back pain symptoms and the lumbar function, respectively.

Results: After transplantation, the pain and function improved immediately in the 2 patients. The VAS and ODI scores decreased obviously during a 2-year follow-up period.

Limitations: The shortcoming of this study is that it is a preliminary study with only 2 patients.

Conclusion: The clinical outcomes indicated that HUC-MSC transplantation is a favorable alternative method for the treatment of chronic discogenic low back pain.

Key words: Intervertebral disc degeneration, discogenic low back pain, chronic low back pain, lumbar discography, mesenchymal stem cells, human umbilical cord mesenchymal stem cells, transplantation

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Chronic low back pain is one of the biggest medical and social problems in the world today (1,2). Discogenic low back pain originating from intervertebral disc degeneration is considered to be one of the major causes of chronic low back pain (3). Current treatment options for this disease are limited to symptomatic treatment, including analgesics, physiotherapy, and minimally invasive

or surgical treatment (spinal fusion or non-fusion), but none of the methods addresses the underlying problem. The pathological process of intervertebral disc degeneration cannot be prevented by these therapies.

The normal disc is a relatively acellular tissue with the average cell density of 5.8×10^3 cells/mm3 that decreases significantly with age, but plays a paramount role in matrix synthesis and maintenance of a healthy tissue (4). Disc degeneration commonly involves changes in disc morphology and composition of the extracellular matrix as well as loss of disc cells. Therefore, a potential therapeutic strategy would be the augmentation of the disc cell population to restore normal biologic function and matrix insufficiencies. A source of such cells with a regenerative potential could be mesenchymal stem cells (MSCs). MSCs can be readily obtained from autologous sources such as bone marrow (BM-MSCs) or adipose tissue (AD-MSCs). This makes MSCs better candidates for transplantation. Increasing evidence has demonstrated that they are capable of differentiating into nucleus pulposus-like cells. Sakai et al (5) demonstrated that MSCs transplanted to degenerative discs in a rabbit model proliferated and differentiated into cells expressing some of the major phenotypic characteristics of nucleus pulposus cells, suggesting that these MSCs may have undergone site-dependent differentiation. Furthermore, implantation of BM-MSCs into a rabbit model of disc degeneration reversed some of the degenerative changes when compared with no treatment (6). Recently, Yoshikawa et al (7) carried out 2 clinical case studies in which MSC transplantation restored disc height and function and improved symptoms. More recently, Orozco et al (8) reported a pilot study in which 10 patients with discogenic back pain underwent injection of autologous expanded MSCs into the nucleus and showed rapid improvement in pain and disability. Both animal and clinical studies indicate MSC therapy is a promising treatment for disc degeneration. Moreover, there are encouraging results reported concerning stem cells obtained from other sources, such as human umbilical cord tissue, that also are capable of differentiating toward mesenchymal cell lineages (9). Comparing human umbilical cord tissue-derived mesenchymal stem cells (HUC-MSCs) with BM-MSCs, HUC-MSCs have been specifically shown to be viable for allogeneic applications due to both their low immunogenicity and their capacity for localized immunosuppresion (10). In addition, HUC-MSCs have many other advantages such as the wide range of sources and the ease of their collection, storage, and transport, and no ethical controversy (11). Recently Wang et al (12) reported that HUC-MSC transplantation significantly improves neurological function in patients with sequelae of traumatic brain injury. However, to the best of our knowledge, there have been no reports on intervertebral disc regeneration therapy using HUC-MSCs in clinical settings. We performed a clinical study using HUC-MSC transplantation in 2 patients with discogenic low back pain to test its feasibility and safety and obtain the early indications of its therapeutic value.

Methods

This study obtained the approval of the medical ethics committee of our hospital. Written informed consent was obtained from the 2 patients before the clinical trial.

Preparation of HUC-MSCs

Informed consent from the parents was obtained for the use of the sample for research purposes. HUC-MSCs were prepared as previously described (12). After the cord was disinfected in 75% ethanol for 30 seconds, the arteries, veins, and epithelium were stripped and discarded from the umbilical cord tissue with surgical tweezers. The cord was cut into fragments of approximately 0.5 cm^3 and centrifuged at 250g for 5 minutes. Following removal of the supernatant, these fragments were washed with serum-free Dulbecco's modified Eagle's medium (DMEM, Gibco) and centrifuged at 250g for 5 minutes. After aspiration of the supernatant, the fragments were placed into a 6-well plate, cultured in DMEM supplemented with 10% FBS, and incubated at 37^0 C in a humidified tissue culture incubator in 5% CO_2 and 95% air. After 10 days in culture, the adherent cells from individual explanted cord tissue sections were observed. The cord tubes were removed from the cultures, and the adherent cells were cultured to 80% confluence. HUC-MSCs at between passages 6 and 8 were used for transplantation. Prior to clinical applications, multiple tests were performed on the HUC-MSCs to ensure the quality of cells. The suspension of l mL HUC-MSCs containing 1×10^7 cells was placed in a 2 mL injector, packaged aseptically, and brought to the operating room for the intradiscal transplantation.

Discography and Diagnosis

Although patients may be symptomatic with some magnetic resonance imaging (MRI) correlations, MRI findings could not determine the origin of low back pain. Lumbar discography was recommended to iden-

tify the pain-generating disc (13,14). Discography was performed under fluoroscopy with 21G needle, using a standard posterolateral approach. Once the needle was accurately inserted into the center of the disc, nonionic contrast medium, Isovist (Schering Ltd, Germany), was instilled slowly into the nucleus under low pressure controlled by hand. A positive discography was defined if patients experienced exact reproduction of their usual pain response pattern, and the posterior annular disruption was shown to extend into the outer annulus or beyond the confines of the outer annulus by the contrast medium. In addition, at least one control disc adjacent to the painful disc was negative. According to the "Modified Dallas Discogram Description" method (15,16), the degrees of annular disruption could be classified into 4 grades. Grades 0, 1, and 2 are normal, while grades 3 and above are indicative of annular disruption. The diagnostic criteria for discogenic low back pain established by the International Association for the Study of Pain (IASP) are emergence of a concordant pain response during discography, internal annular disruption shown by computed tomograph (CT) after discography (CTD), and at least one adjacent disc without concordant pain (17).

Case 1

The patient was a 45-year-old woman with a history of low back pain for 2 years without lower leg pain. She failed extensive conservative therapies including physical therapy, exercise, and drug therapy. Physical examination revealed a stiff lumbar spine with limited range of flexion, extension, and lateral bending. There were tenderness and percussion pain over the L4-L5 lumbar spine. Muscle strength, dermatomal sensation, and deep tendon reflexes in lower extremities were all normal. Lumbar radiographs showed lumbar lordosis disappearance and normal height of all discs. Her lumbar MRI scan showed L3/4, L4/5, and L5/S1 disc degeneration without disc herniation (Fig. 1). Laboratory examinations including blood cell count, liver enzymes, erythrocyte sedimentation rate, C-reactive protein, HLA-B27, and rheumatoid factor were either negative or within normal range.

The patient underwent provocative discography at the lower 3 lumbar discs (Fig. 2). Although both L3/4 and L5/S1 discs showed grade 5 disruption during discography, the patient did not experience any pain response. The result of discography showed L4/5 disc grade 5 disruption with pain reproduction, which indicated the L4/5 disc as the source of pain. Accordingly, the transplantation of HUC-MSCs was performed at L4/5

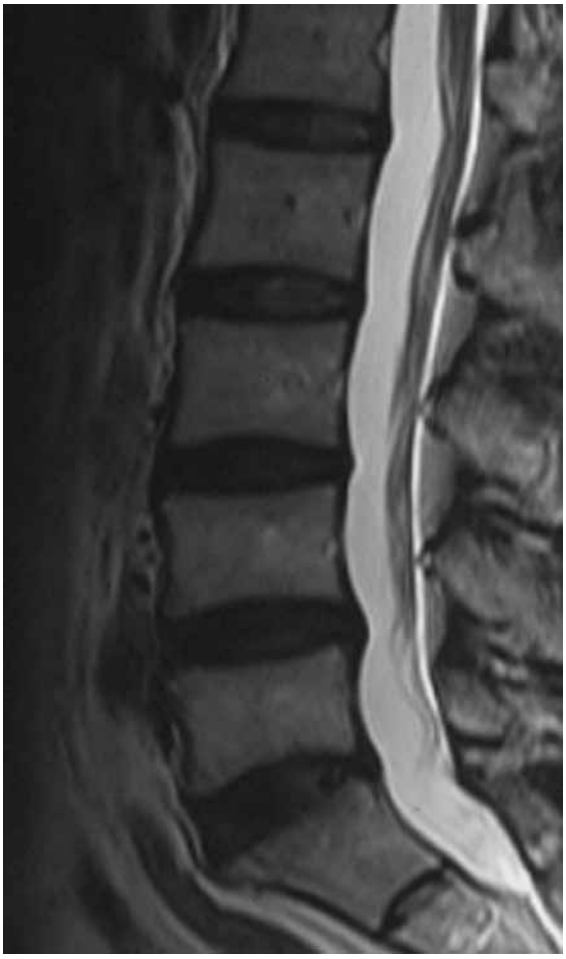

Fig. 1. *T2-weighted MRI showing L3/4, L4/5, and L5/S1 disc degeneration.*

disc through the discographic needle immediately following discography. After the procedure, intravenous cephalosporin antibiotic immediately was used.

This procedure was successfully performed and the patient reported obvious back pain relief and lumbar function improvement. The alleviation of pain and improvement in physical function was assessed by the change in the degree of pain with a self-assessment of pain by an 11-point Visual Analog Scale (VAS, 0 – 10) pain scales and the Oswestry Disability Index (ODI, version 1.0, 0 – 100) (18). The VAS score was 7 before HUC-MSC transplantation, and was 2, 1, and 1 at 6, 12, and 24 months, respectively, after transplantation. The ODI was 46 before HUC-MSC transplantation, and was 10, 5, and 5 at 6, 12, and 24 months, respectively, after transplantation. At 2 years after the procedure,

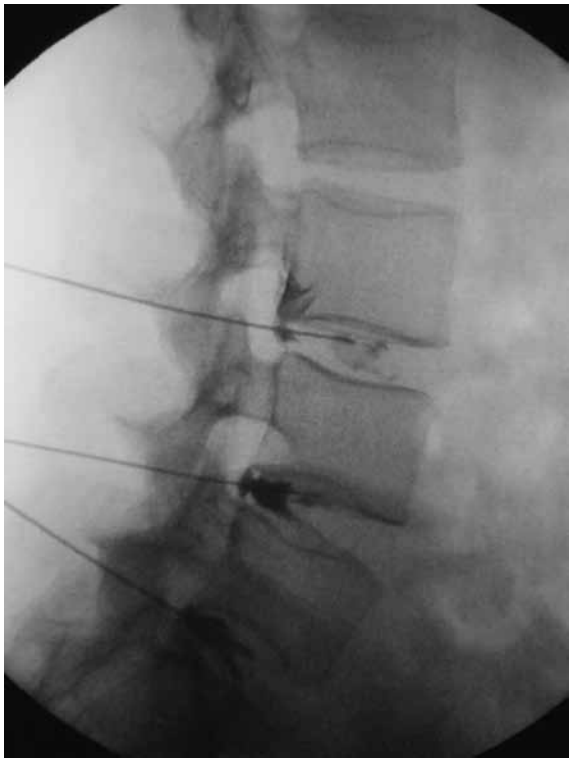

Fig. 2. *Discography showing lower 3 lumbar disc grade 5 disruption, but only L4/5 disc accompanied with pain reproduction response.*

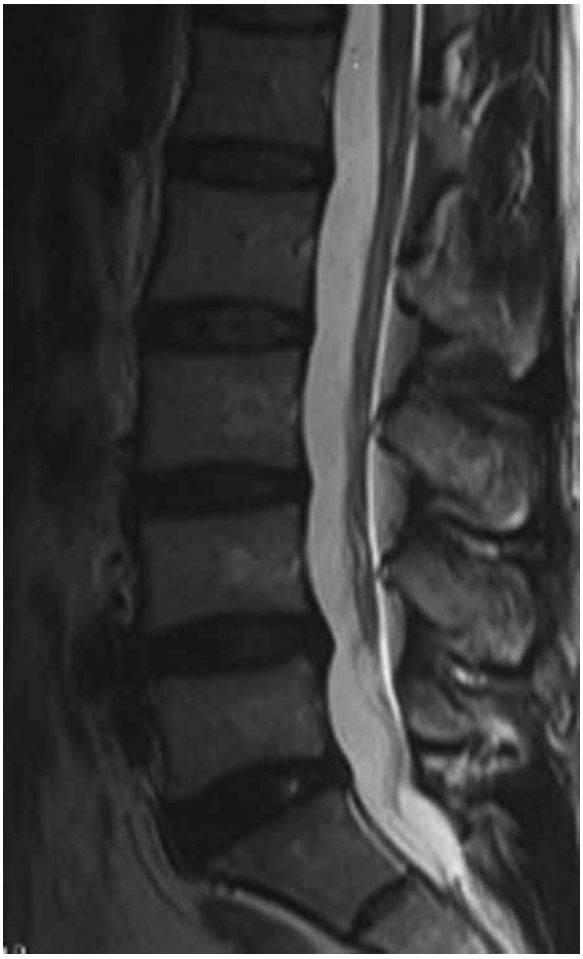

Fig. 3. *T2-weighted MRI at 2 years after transplantation showed increased signal intensity in L4/5 disc.*

T2-weighted MRI signal intensity in the L4/5 disc was higher when compared with that before transplantation, which indicated higher water content in the nucleus pulposus of L4/5 disc (Fig. 3). No adverse events were found in the patient.

Case 2

The patient was a 38-year-old man with a history of persistent low back pain for 4 years. He was nonresponsive to conservative therapies. His plain radiographs showed a almost normal lumbar spine. His lumbar MRI revealed L3/4 disc degeneration, and did not show any disc herniation. Physical examination showed tenderness over the L3-L4 lumbar spine. Laboratory examinations were all normal.

The patient underwent provocative discography at L3/4 and L4/5 discs. The discography revealed L3/4 disc grade 5 disruption with pain reproduction, which indicated the L3/4 disc as the source of back pain. Subsequently, the HUC-MSCs were grafted in the L3/4 disc as in case 1. After transplantation, the pain and function improved immediately. VAS scores improved from 8 before transplantation to 2, 3, and 4, respectively, at 6, 12, and 24 months after the procedure. ODI scores decreased from 56 before transplantation to 10, 15, and 20, respectively, at 6, 12, and 24 months after the procedure. Although there were obvious pain relief and function improvement in the patient, no notable increase of T2-weighted MRI signal intensity of the L3/4 disc was found. No adverse events occurred in the patient.

Discussion

Due to proliferative potential and multidifferentiation capacity, adult MSCs provide an attractive choice for managing disc degeneration. However, the harvest of adult MSCs is a highly invasive procedure. Moreover,

the BM-MSCs represent only a small percentage of the total number of cells in bone marrow, and the number of cells useful for regenerative medicine applications is extremely low (19) and the yield of MSCs from bone marrow also significantly decreases with donor age (20,21). Umbilical cord, an ecto-embryo tissue, may be an ideal source of stem cells because of its accessibility, abundant resources, painless procedures for harvesting, and lack of ethical issues (22). HUC-MSCs are multipotent and can be induced to differentiate to various cell types such as cardiomyocyto, osteogenic, adipogenic cell, neural cell, and myogenic cell under suitable culture conditions (22). Chon et al (23) demonstrates that HUC-MSCs have the potential to differentiate into immature nucleus pulposus-like cells within a special culture system. A recent study indicated that HUC-MSCs could also be induced to differentiate to nucleus pulposus-like cells by coculturing with nucleus pulposus cells (22). These studies suggested that HUC-MSCs could be differentiated into nucleus pulposus-like cells after being grafted into a degenerative disc, and thus could restore the extracellular matrix.

The present study showed that HUC-MSC transplantation is both feasible and safe, with no side effects. The analgesic effect of treatment was remarkable using HUC-MSC transplantation. The improvement in back pain was accompanied by a parallel improvement in lumbar function. In addition, we also found that the water content in the degenerative painful disc in case 1 was significantly increased at 2 years after transplantation. The underlying mechanisms of the treatment remain unclear. Recent data from animal studies have shown changes in cytokine expression following growth factor injection, indicating a possible mechanism for pain relief (24). HUC-MSCs may also help relieve pain by reducing inflammation. A recent study indicates that MSCs also induce the production of anti-inflammatory cytokines (25). The increase of water content in the painful disc in case 1 indicated that HUC-MSCs could induce the synthesis of proteoglycans and restore disc structure.

Treatment for chronic discogenic low back pain has traditionally been limited to either conservative management or surgical fusion. If conservative treatment fails, then surgical fusion is commonly considered. During recent decades, surgical fusion of the lumbar spine has been performed in increasing numbers on patients with chronic low back pain. However, the reported results vary considerably in different studies, and the complication rate after fusion surgery in the lumbar spine is not negligible (26). The alternative cell-based therapy proposed here avoids these side effects and is a simpler, less-invasive intervention.

However, disc degeneration is complex and its regeneration represents a significant challenge. The normal nucleus pulposus has an acidic pH, low oxygen tension, and paucity of basic nutrients. To survive in this harsh environment, disc cells are highly specialized (27). The survival of the transplanted HUC-MSCs could be a limiting factor. In the future, these transplanted cells may have to be preconditioned, possibly by genetic manipulation. In addition, the leakage of transplanted HUC-MSCs through the annular tears should be prevented. Further, transplantation with a suitable biomaterial scaffold could be required when injecting these cells to a painful disc. With continued dedication, we believe that the disc regeneration therapy will someday play a major role in the treatment of disc degenerative disease.

Conclusion

The present study indicates that HUC-MSC transplantation is a favorable alternative method for the treatment of chronic discogenic low back pain. Further studies will be needed with a large sample size and longer follow-up time.

Acknowledgments

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